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(71) Applicant: THE GOVERNMENT OF THE UNITED STATES, represented by THE SECRETARY OF THE NAVY [US/US]; Naval Research Laboratory, 4555 Overlook Avenue, S.W., Washington, DC 20375-5325 (US).

(72) Inventors: LIGLER, Frances, S.; 12400 Beall Mountain Road, Potomac, MD 20854-1121 (US). SHRIVER-LAKE, Lisa, C.; 11869 Weller Hill Drive, Monrovia, MD 21770 (US). WIJESURIYA, Dayaweera, C.; Apartment 1105, 3220 Rue Voltaire, South Bend, IN 46615 (US).

(74) Agent: McDONNELL, Thomas, E.; Naval Research Laboratory, Associate Counsel (Patents), Building 28, Room 260, Code 3008.2, 4555 Overlook Avenue, S.W., Washington, DC 20375-5325 (US).

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(54) Title: OPTICAL IMMUNOASSAY FOR MICROBIAL ANALYTES USING NON-SPECIFIC DYES

(57) Abstract

The presently disclosed invention relates to a method of rapid detection and identification of microorganisms including bacteria, viruses, rickettsiae and fungi. The method involves staining all microorganisms or fragments thereof in a sample. The stained sample is introduced onto a surface coated with a capture molecule specific for the microorganism of interest, and the bound microorganism or fragment thereof is then optically detected. For example, detection of *B. anthracis* and *Salmonella* was achieved in times of approximately one minute. The sensitivity of this method is on the order of about 3 cells/μl.

OPTICAL IMMUNOASSAY FOR MICROBIAL ANALYTES USING NON-SPECIFIC DYES

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to a method for detecting the suspected presence of a microbial analyte within a sample. More specifically, the present invention relates to an optical detection method using non-specific dyes for detecting the suspected presence of microbial analytes, for example, bacteria, viruses, fungi, rickettsiae and fragments of these microbial analytes among others.

DESCRIPTION OF THE RELATED ART

There is a requirement for rapid methods of detection and identification of microbial analytes, for example, microorganisms, bacteria, viruses, rickettsiae, fungi and their fragments not only for medical diagnosis, but also for agriculture, food processing, bioprocessing and water purification. Current methods include cell culture, microscopy, immunoassay and nucleic acid probes. Assay times vary from days to minutes. Only culture and polymerase chain reaction (PCR) based tests are very sensitive. Culture and microscopy depend on the isolation of the intact microorganisms from the milieu to be tested, and, for culture, the cells must be viable. Tests based on genetic methods, including polymerase chain reaction, require intact deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The tradeoffs inherent in these various methods are summarized below.

In the immunoassay and immunofluorescence stains previously described, a complex is formed between the antibody, the analyte recognized (from or on the microorganism) and a label or signal generator (i.e. enzyme) that can be measured. The measurement may represent the formation of such a complex, as in sandwich immunoassays, or the lack of formation of such complexes, as in most competitive immunoassays. In a sandwich immunoassay, the label or signal generator is attached to an antibody. There is no direct attachment of the label to the analyte. The binding of the label or signal generator to the analyte is via the antibody.

In a competitive assay, the label or signal generator is bound to an antigen similar to the analyte. As the analyte competes with the labeled antigen for binding to the antibody, the amount of signal changes. In this case also, the label never directly attaches to the analyte.

Other binding molecules besides antibodies have been demonstrated to be useful in sandwich and competitive assays. Such binding molecules include, but are not limited to, lectins, deoxyribonucleic acid (DNA) binding proteins listed by D.J.

Furthermore, all of the above patents disclose the use of dyes attached to specific binding molecules (i.e. antibodies or antigens). Assays using dyes bound to specific binding elements may require an incubation time, may be very limited in the amount of the dye that is actually associated with the analyte, and may introduce multi-step procedures into the assay.

In addition to immobilization of antibodies for assay purposes, antibodies on solid supports have been used to bind intact cells and remove them from complex mixtures containing other cell types. Most commonly used forms of this approach are affinity chromatography and panning. In both cases, the sample, containing mixtures of cells, is incubated with the antibody-coated solid support and then the support is gently but thoroughly washed. The bound cells are eluted and subsequently may be subjected to a variety of characterization or experimental procedures. Affinity chromatography and panning techniques are used for isolation purposes rather than for detection of microorganisms or microbial analytes. Such techniques are relatively time consuming, inefficient in terms of cell recovery, and require technical training.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to detect the presence of a microbial analyte in a rapid, simple manner.

It is another object of the present invention to detect the presence of a microbial analyte without the use of dyes attached to specific binding molecules.

It is yet another object of the present invention to incorporate a large number of dye molecules in each complex of the dye, the microbial analyte and the capture molecules.

and FDF-1B9, an antibody specific for *B. anthracis*. Curve B was produced by the complex of Nile red and *B. anthracis* in the presence of an optical fiber coated with goat immunoglobulin G (goat IgG). Curve C was produced by the complex of Nile red and *B. anthracis* in the presence of an optical fiber coated with rabbit anti-goat immunoglobulin G (rabbit anti-goat IgG). FIG. 2 is schematic representation of a stained microbial analyte 30 attached to a capture molecule 20 attached to optical fiber 10 referred to in EXAMPLE 2.

FIG. 3 is a plot similar to that in FIG. 1. However, the microbial analyte (*B. anthracis*) concentration has been decreased. Curve A was produced by the complex of Nile red, *B. anthracis* and FDF-1B9, an antibody specific for *B. anthracis*. Curve B was produced by the complex of Nile red and *B. anthracis* in the presence of an optical fiber coated with mouse immunoglobulin G (goat IgG). Curve C was produced by the complex of Nile red and *B. anthracis* in the presence of an optical fiber coated with rabbit anti-goat immunoglobulin G (rabbit anti-goat IgG).

FIG. 4 is a plot of microbial analyte concentration versus optical signal, measured in microvolts (μV), produced by a complex, attached to an optical fiber, of Nile red, microbial analyte and a capture molecule, an antibody, referred to in EXAMPLE 5. Curve A was produced by the complex of Nile red, *B. anthracis* and FDF-1B9. Curve B was produced by the complex of Nile red and *B. subtilis* in the presence of an optical fiber coated with FDF-1B9.

FIG. 5 is a bar graph representation of trial number (no. of uses) versus optical signal, measured in μV, over background produced by a complex, attached to an optical fiber, of Nile red, *B. anthracis* and FDF-1B9 referred to in EXAMPLE

FIG. 9 is a plot of time versus optical signal, measured in μV, produced by a complex, attached to an optical fiber, of Nile red, *B. anthracis* and FDF-1B9 referred to in **EXAMPLE 3.**

DETAILED DESCRIPTION OF THE INVENTION

The following detailed description of the preferred embodiment is provided to aid those skilled in the art in practicing the present invention. However, the following detailed description of the preferred embodiment should not be construed to unduly limit the present invention. Variations and modifications in the embodiments discussed may be made by those of ordinary skill in the art without departing from the scope of the present inventive discovery.

For purposes of consistent usage of terminology, a microbial analyte is defined as a microorganism or fragments thereof which are uniquely associated with a specific microorganism or class of microorganisms. Thus, a microbial analyte includes, for example, the following: a bacteria or fragments thereof, a virus or fragment thereof, a fungi or fragments thereof, rickettsiae or fragments thereof among others.

With respect to microbial analytes that are bacteria or fragments thereof, fungi or fragments thereof, viruses or fragments thereof, rickettsiae or fragments thereof, they include, but are not limited to, the following:

Mitoplasma capsulatum, Cryptococcus neoformans, Blastomyces dermatitidis, Paracoccidioides brasilenensis, Coccidioides immitis, Corynebacterium diphtheriae, Corynebacterium heamolyticum, Corynebacterium pseudodiphtheriticum, Corynebacterium pseudotuberculosis, Corynebacterium ulcerans, Corynebacterium xerosis, Bacillus anthracis, Bacillus thurengensus, Pneumocistis pneumoniae,

Type Culture Collection, CATALOGUE OF VIRUSES, ANIMAL & PLANT, RICKETTSIAE AND CHLAMYDIAE, Seventeenth edition, Gherna and Pienta, Eds., (Rockville, Md., 1989), each reference incorporated herein by reference in its entirety and for all purposes. Other microbial analytes are listed in M.J. Pelczar, Jr. and R.D. Reid, MICROBIOLOGY, McGraw-Hill Book Company, New York (1972), incorporated herein by reference in its entirety and for all purposes.

To a given sample of material suspected of containing a microbial analyte of interest (for example, a bacteria or fragments thereof, viruses or fragments thereof, fungi or fragments thereof and rickettsiae or fragments thereof) a non-specific dye which stains biological elements is added. A non-specific dye is a dye which specifically labels neither the microbial analyte, a binding element specific to the microbial analyte, nor an analog of the analyte that competes with the analyte for binding to a binding element specific to the analyte. A biological element of a microbial analyte includes, but is not limited to, membranes, cell walls, DNA, RNA, cytoskeletal structures, mitochondria and fragments thereof. In the preferred embodiment of the invention, the dye is fluorescent. Dyes which label, for example, metals, carbohydrates, nucleic acids, lipids or proteins can be used. Several of these dyes are given by G.L. Humason in ANIMAL TISSUE TECHNIQUES, 4th Ed. (1979), incorporated herein by reference in its entirety and for all purposes. Another preference is for dyes that have low fluorescence in aqueous media, but fluoresce when intercalated into microorganisms (including the microbial analyte of interest) and their fragments. Dyes that fluoresce at wavelengths where the unstained sample exhibits relatively low levels of fluorescence are also preferred. Suitable non-specific dyes may be selected from those used to stain lipids or DNA

include two classes of dyes including those dyes with hydroxyl groups and those dyes without hydroxyl groups. The anionic moderately or strongly amphoteric dyes further include three classes of dyes including those dyes without hydroxyl groups, those dyes with hydroxyl groups and those dyes with hydroxyl groups as their only acidic colligators. Numerous examples of these nonionic cationic and anionic dyes are given by **Edward Gurr** and are suitable as non-specific dyes for use in conjunction with the present inventive process published in SYNTHETIC DYES IN BIOLOGY, MEDICINE AND CHEMISTRY, **Academic Press, (London & New York, 1971)**, incorporated herein by reference in its entirety and for all purposes.

Once microbiological material within a sample suspected of containing the microbial analyte is stained using a non-specific dye, the stained sample is introduced over a solid support coated with a capture molecule specific for the microbial analyte of interest. The capture molecule can be adsorbed or covalently bound to the solid support. One procedure for immobilizing capture molecules onto a solid support material is given in **U.S. Patent No. 5,077,210** of **Ligler et al.**, incorporated herein by reference in its entirety. Of course, other immobilization procedures may be used in order to optimize these procedures to maintain the binding function of the capture molecule. the method used for immobilization is not critical to the present invention. Capture molecules include but are not limited to antibodies, lectins, cell receptors, DNA binding proteins or specifically engineered peptides referenced in ***Random Peptide Libraries: A Source of Specific Protein Binding Molecules*** by **J.J. Devlin et al.** published in SCIENCE, **Vol. 24, pp. 404-405 (1990)**, incorporated herein by reference in its entirety and for all

nm of the fiber core) of the optical fiber. Antibody- based sensing using evanescent wave detection has been carried out by **Shriver-Lake, Anderson, Golden and Ligler** as described in ***The Effect of Tapering the Optical Fiber on Evanescent Wave Measurements*** and published in ANALYTICAL LETTERS, **25(7), 1183-1199 (1992),** incorporated herein by reference in its entirety and for all purposes. In addition, fiber optic probes have been carefully fashioned to achieve both optimal excitation of the bound fluorophore and capture of its subsequent emission. ***See*** **L.C. Shriver-Lake, G.P. Anderson, G.P. Golden and F.S. Ligler,** ***The Effect of Tapering the Optical Fiber on Evanescent Wave Measurements***, ANAL. LETT. **25 (7): 1183-1199 (1992),** incorporated herein by reference in its entirety and for all purposes; ***See*** **G. P. Anderson, J.P. Golden and F.S. Ligler,** ***Fiber Optic Biosensor: Combination Tapered Fibers Designed for Improved Signal Acquisition***, BIOSENSORS & BIOELECTRONICS **8 (1993),** incorporated herein by reference in its entirety and for all purposes.

Dyes can be used which generate, for example, visible, fluorescence, luminescence, colorimetric, infrared (IR) and ultraviolet (UV) signals. The methods of measuring the dye-microbial analyte-capture molecule complexes include, for example, spectroscopy, microscopy, visual detection, electron spin resonance, phosphorescence and optical waveguide detection.

The following examples outline preferred embodiments of the present invention.

EXAMPLE 1

Choice of Stain for Assay

To select a dye appropriate for use with the microbial analyte of interest and the optical readout system selected by the user, it must be determined that the dye

STAINING DYE	MOLAR CONCENTRATION	STAINING INTENSITY
Propidium iodide	8.5×10^{-4}	++++
Propidium iodide	8.5×10^{-6}	++++
Propidium iodide	8.5×10^{-7}	+++
Propidium iodide	8.5×10^{-8}	+
Ethidium bromide	1.7×10^{-3}	++++
Ethidium bromide	1.7×10^{-5}	++++
Ethidium bromide	1.7×10^{-6}	+++
Ethidium bromide	1.7×10^{-7}	+
Acridine orange	1.2×10^{-3}	++++
Acridine orange	1.2×10^{-5}	-
Acridine orange	1.2×10^{-6}	-
Acridine orange	1.2×10^{-7}	-
Nile red	2.2×10^{-5}	++++
Nile red	2.2×10^{-6}	++++
Nile red	2.2×10^{-7}	+++
Nile red	2.2×10^{-8}	+
D-384	3.27×10^{-5}	++++
D-384	3.27×10^{-7}	++
D-384	3.27×10^{-8}	-
D-384	3.27×10^{-9}	-

EXAMPLE 2

Detection of Nile Red-Stained Microbial Analytes

The mouse monoclonal antibody, FDF-1B9, specific for capsular material of *B. anthracis* vegetative cells (obtained from Dr. John Ezzell, US Army Institute of Infectious Diseases, Ft. Detrick, Maryland) was received as ascites. Immunoglobulin G (IgG) fractions were prepared using protein G-agarose. Protein

Thiol-Terminal Silanes and Heterobifunctional Crosslinkers for Immobilization of Antibodies on Silica Surfaces, ANAL. BIOCHEM., 178, 408-413, (1989), incorporated herein by reference in its entirety and for all purposes. The latter reference discloses the use of thiol-terminal silanes and heterobifunctional crosslinkers for immobilization of antibodies on silica surfaces.

The fluorimeter portion of the fiber optic biosensor consists of a 50 mW argon-ion laser, an off-axis parabolic mirror and spherical lens. The laser beam (514 nm) passed through the off-axis parabolic mirror, and was focused by the spherical lens onto the proximal end of the fiber. A chopper was placed between the spherical lens and the fiber and interfaced to a lock-in amplifier for phase-sensitive detection. The collected fluorescence signal traveled the reverse path to the parabolic mirror where it was refocused through a bandpass filter (KV550) onto a silicon photodiode which was also connected to the lock-in amplifier. Data was collected using a lap-top computer. A detailed description of this fiber optic fluorimeter can be found in **G.P. Anderson, L.C. Shriver-Lake, J.P. Golden, F.S. Ligler,** ***Fiber Optic-Based Biosensor: Signal Enhancement in a Production Model,*** **SPIE JOURNAL, 1648, 39-43 (1992)**, incorporated herein by reference in its entirety and for all purposes.

The antibody-coated region of an optical fiber was mounted in a glass capillary tube using T-connectors at both ends. The distal end of the fiber was blocked with a non-fluorescent glue.

A dose/response curve of demonstrating the capability of the assay to detect Nile red-stained *B. anthracis* (vegetative cells) was obtained as follows:

response) value calculated from the response/time curve was on the order of 35 seconds.

EXAMPLE 4

Demonstration of Assay Sensitivity

Sensitivity and possible detection limits of the assay were investigated for the FDF-1B9 antibody and ***B. anthracis*** cells stained using Nile red and using the fiber optic biosensor as the readout system as described in **EXAMPLE 2.**

Dose/response curves (**Figure 3**) of the assay for stained cells (concentrations ranging from 3 to 50 cells/μl) were obtained as described in **EXAMPLE 3.** Lowest detection limit of the assay obtained with this particular antibody-cell pair and under these particular conditions was 3 cells/μl or about 1000 cells. The volume of the reaction chamber into which the fiber was inserted was 325 μl. The use of larger volumes or improvements in the biosensor itself might lower the limits of sensitivity even further.

EXAMPLE 5

Demonstration of Assay Selectivity

Selectivity of the assay was evaluated using the FDF-1B9 antibody, ***B. anthracis*** and a similar bacteria ***B. subtilis***, Nile red stain, and the fiber optic biosensor. The assay procedure was performed as in **EXAMPLE 2**, with respect to the FDF-1B9 antibody coated fiber.

Response of the sensor for Nile red-stained ***B. anthracis*** (vegetative form, **Figure 4: curve A**) and ***B. subtilis*** (vegetative form, **Figure 4: curve B**) were compared as shown. Fibers coated with specific antibodies to ***B. anthracis*** (FDF-1B9) were exposed to Nile red-stained ***B. anthracis*** and ***B. subtilis*** cells at increasing

river water and serum, respectively. In **Figure 6**, curve A was produced by a complex of Nile red, *B. anthracis* and FDF-1B9. Curve B was produced by a complex of Nile red and *B. anthracis* in the presence of an optical fiber coated with mouse immunoglobulin G (mouse IgG). In **Figure 7**, curve A was produced by a complex of Nile red, *B. anthracis* and FDF-1B9. Curve B was produced by a complex of Nile red and *B. anthracis* in the presence of an optical fiber coated with mouse immunoglobulin G (mouse IgG).

EXAMPLE 8

Detection of Ethidium Bromide-Stained *Salmonella* Cell Fragments Using Microscopy

Salmonella and an antibody specific for its flagella were provided by Dr. Eleanor Metcalf, Uniformed Services University of the Health Sciences (Bethesda, Maryland). The antibody was immobilized on acid-cleaned microscope slides using the procedure of **EXAMPLE 2**. The antibody-coated slides were stored in PBS at pH 7.4 until use.

A stock solution of 0.1 mg/ml ethidium bromide in PBS at pH 7.4 buffer was prepared. In an Eppendorf tube, 90 μl of the *Salmonella* cell sample was mixed with 10 μl of the ethidium bromide solution. The mixture was allowed to incubate for 5 minutes, then the solution was centrifuged in an Eppendorf tube for 1 minute. The supernatant was removed, leaving the cell pellet. The cells were diluted to a known concentration in PBS at pH 7.4 with 2 mg/ml bovine serum albumin (BSA) for exposure to an antibody-coated microscope slide. The stained cell sample was incubated with the antibody-coated microscope slide for 1 hr, then rinsed with PBS at pH 7.4 with rapid shaking. The shaking caused the cells to break apart leaving

complex of Nile red and *Salmonella* in the presence of an optical fiber coated with non-specific immunoglobulin G (IgG).

Corynebacterium diphtheriae, Corynebacterium heamolyticum, Corynebacterium pseudodiphtheriticum, Corynebacterium pseudotuberculosis, Corynebacterium ulcerans, Corynebacterium xerosis, Bacillus anthracis, Bacillus thurengensus, Pneumocistis pneumoniae, Treponema pallidum, Treponema pertenue, Treponema carateum, Leptospira interrogans, Borrelia recurrentis, Borrelia burdorferi, Legionella pneumophila, Legionella micdadei, Legionella bozemanii, Legionella dumoffii, Legionella gormanii, Legionella longbeacheae, Legionella jordanis, Rickettsiae rickettsii, Rickettsiae prowazekii, Rickettsiae mooseri, Rickettsiae tsutsugamushi, Rickettsiae akari, Coxiella burnetii, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio vulnifcus, Vibrio hollisae, Vibrio mimicus, Vibrio fluvialis, Vibrio damsela, Vibrio metschnikovii, Aeromonas hydrphila, Plesiomonas shigelloides, Salmonella typhi, Salmonella enteriditis, Arizona hinshawii, Edwardsiella tarda, Shigella dysenteriae, Shigella flexneri, Shigella boydii, Shigella sonnei, Yersinia enterocolitica, Yersinia pseudotuberculosis, Yersinia pestis, Escherichia coli, Neisseria meningitidis, Nesseria gonorrhoeae, Hemophilus influenzae, Bordetella pertussis, Trypanosoma cruzi, Plasmodium vivax, Leishmania spp., Histoplasma capsulatum, Schistosoma mansoni, Trichinella spiralis, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus epidermidis, Staphylococcus albus, Equine encephalitis virus, Rabies virus, Jungle yellow fever virus, Tomato bushy stunt virus, Tobacco mosaic virus, Alfalfa mosaic virus, rotaviruses, parvoviruses,

10. The method of claim 1 wherein said dye comprises a cationic membrane probe.

11. The method of claim 1 wherein said dye comprises a neutral membrane probe.

12. The method of clain 1 wherein said dye comprises an isothiocyanate stain.

13. The method of claim 1 wherein said dye comprises a protein stain.

14. The method of claim 1 wherein said dye comprises a non-specific stain.

15. The method of claim 1 wherein said dye is selected from the group consisting of:

(a) Nile red;

(b) propidium iodide;

(c) ethidium bromide;

(d) acridine orange;

(e) D-384; and

(f) mixtures thereof.

16. The method of claim 1 wherein said capture molecule specific for said microbial analyte is selected from the group consisting of:

(a) antibodies;

(b) lectins;

(c) cell receptors;

(d) DNA binding proteins;

(e) specifically engineered peptides; and

(f) mixtures thereof.

(a) adding a dye to non-selectively stain biological elements in said sample, thereby, forming a stained sample;

(b) binding antibodies specific for the bacteria or a fragment thereof to a solid support;

(c) exposing said stained sample to said antibodies attached to said solid support; and

(d) optically measuring the formation of said complex.

23. The method of claim 22 wherein said optically measuring step further comprises the step of:

(a) exciting said complex; and

(b) detecting a fluorescence signal.

24. The method of claim 22 wherein said dye is selected from the group consising of:

(a) Nile red;

(b) propidium iodide;

(c) ethidium bromide;

(d) acridine orange;

(e) D-384; and

(f) mixtures thereof.

25. The method of claim 1 wherein said optically measuring step further comprises optically measuring said complex by microscopy.

26. The method of claim 1 wherein said optically measuring step further comprises optically measuring said complex by visual detection.

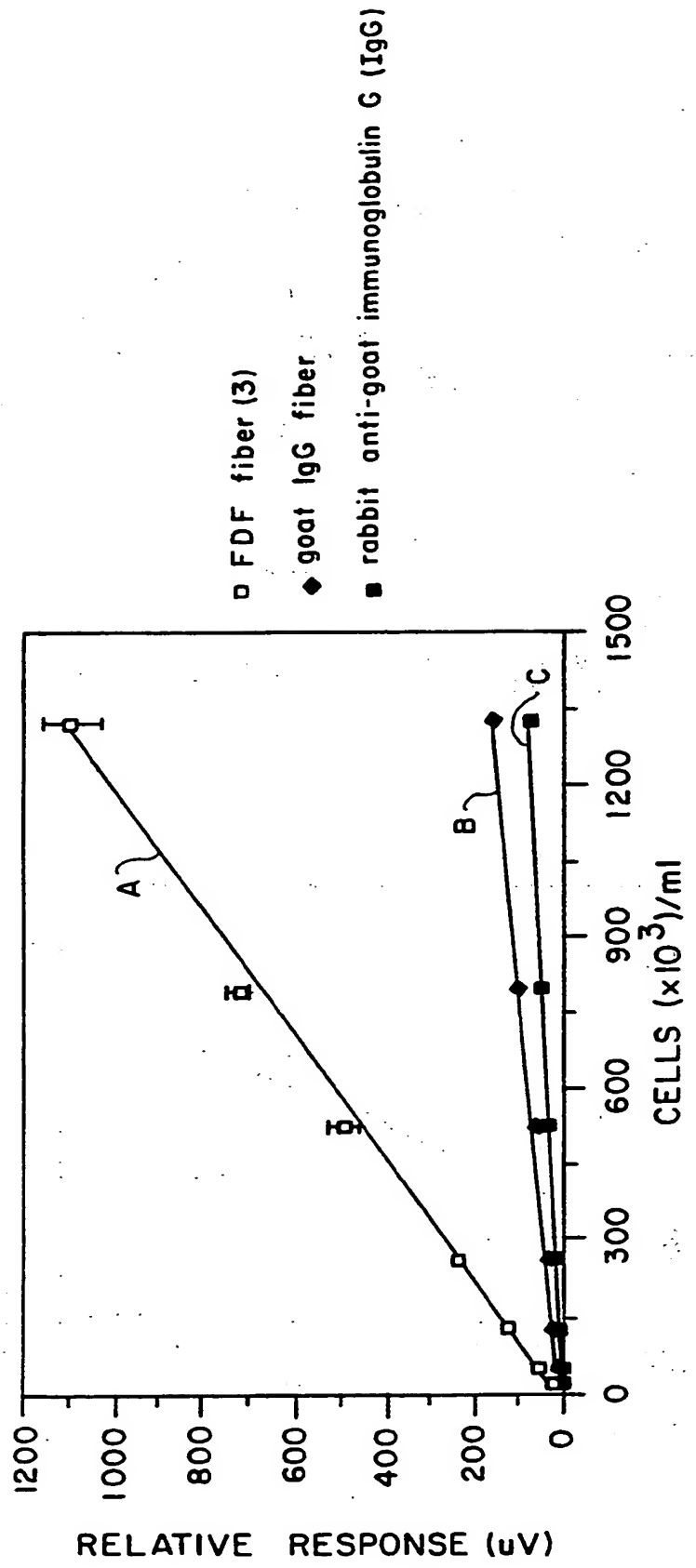

FIG. 1

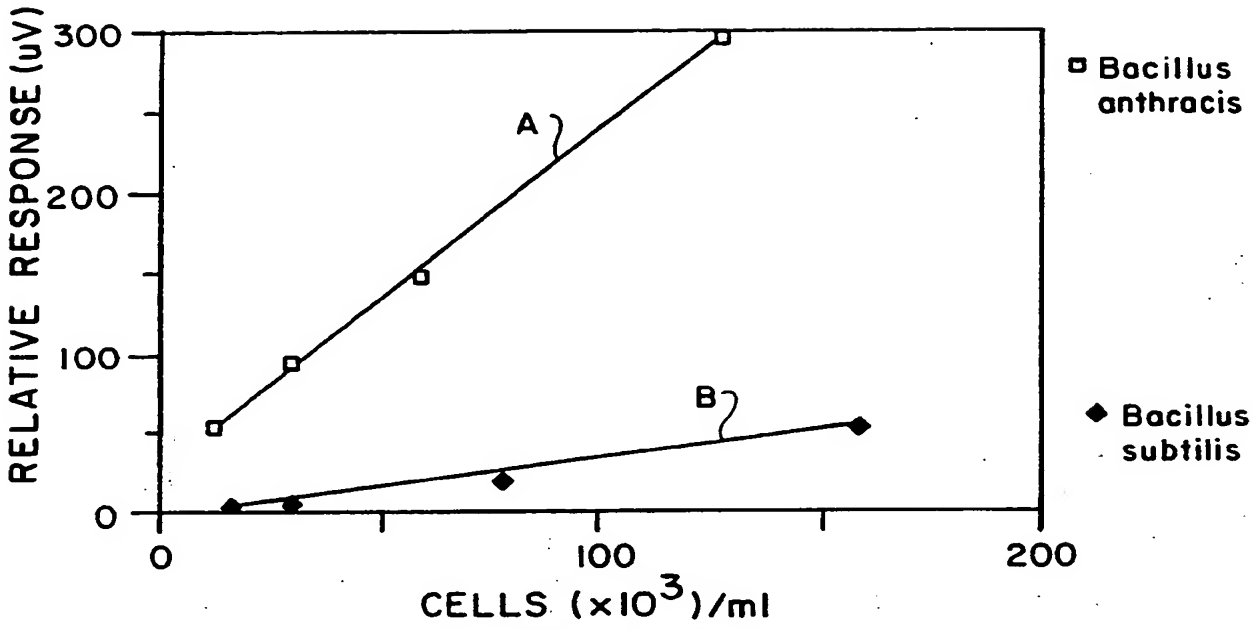

FIG. 4

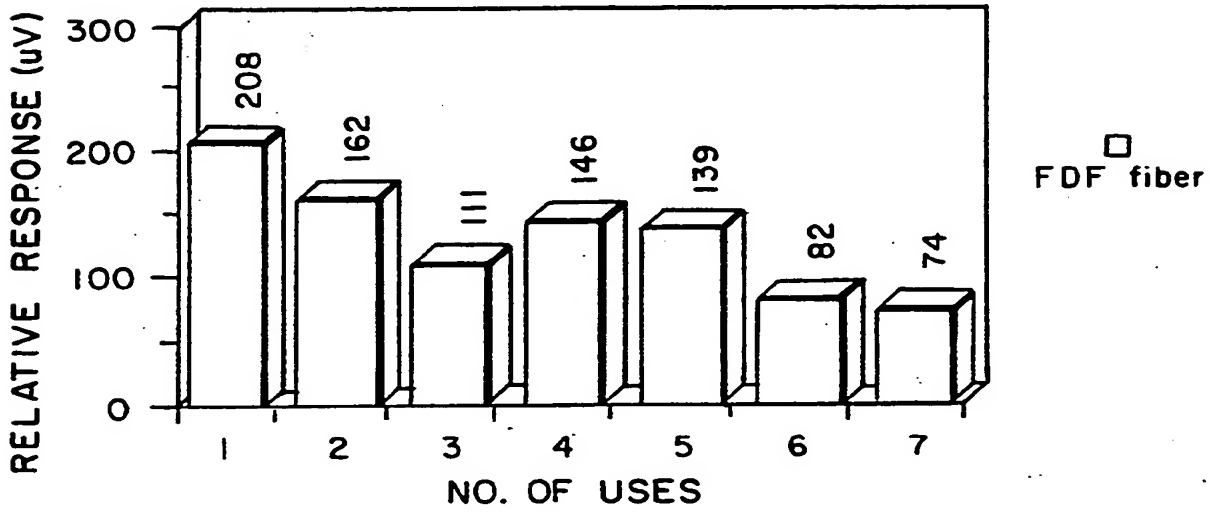

FIG. 5

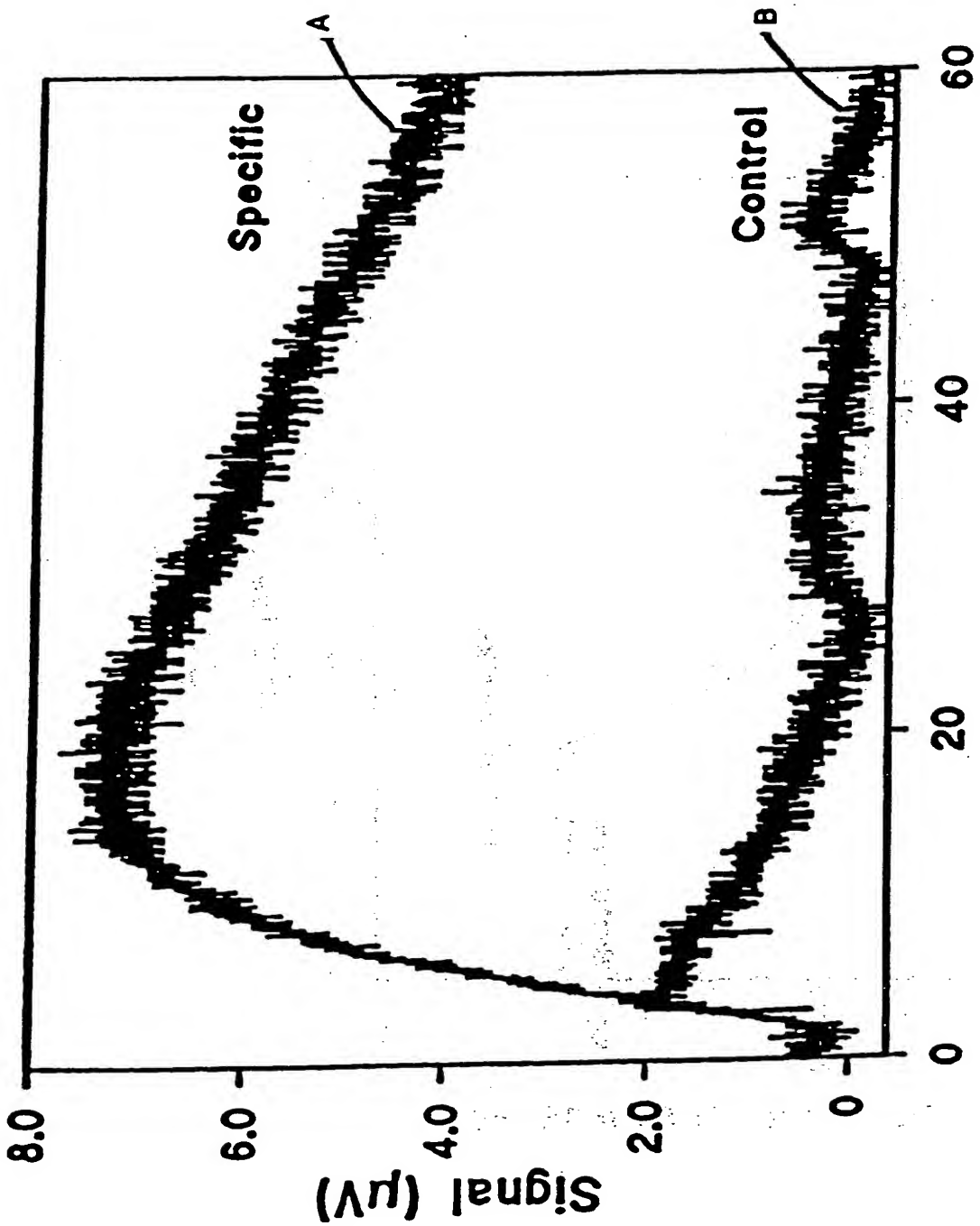

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08752

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/53, 33/567, 33/569; C12Q 1/00, 1/68, 1/70
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 5, 6, 7.1, 7.2, 7.21, 7.22, 7.32, 7.3; 436/501, 518, 519, 528, 800, 824

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,591,570 (CHANG) 27 MAY 1986, column 3, lines 31-68 and column 4, lines 1-38.	1-28
Y	Journal of Immunological Methods, Volume 137, issued 1991, J.M.C. Luk et al, "Rapid and Sensitive Detection of *Salmonella* (O:6,7) by Immunomagnetic Monoclonal Antibody-Based Assays", pages 1-8, especially the right-hand column of page 2.	1-28
Y	Analytical Letters, Volume 25, No. 7, issued 1992, L.C. Shriver-Lake et al, "The Effect of Tapering the Optical Fiber on Evanescent Wave Measurements", pages 1183-1199, especially pages 1190 and 1191.	27

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer TONI R. SCHEINER Jill Warden for Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

Inte...ational application No.
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A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/4, 5, 6, 7.1, 7.2, 7.21, 7.22, 7.32, 7.3; 436/501, 518, 519, 528, 800, 824

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG (file biochem), APS
Search Terms: prestain, pre-stain, antibod?, dye?, stain?, bacteria?, virus?, lectin, nile red, ethidium bromaide, propidium iodide, acridine orange, D-384, fluoresceen?